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Thermal stability of carboxymethyl chitosan varying the degree of substitution

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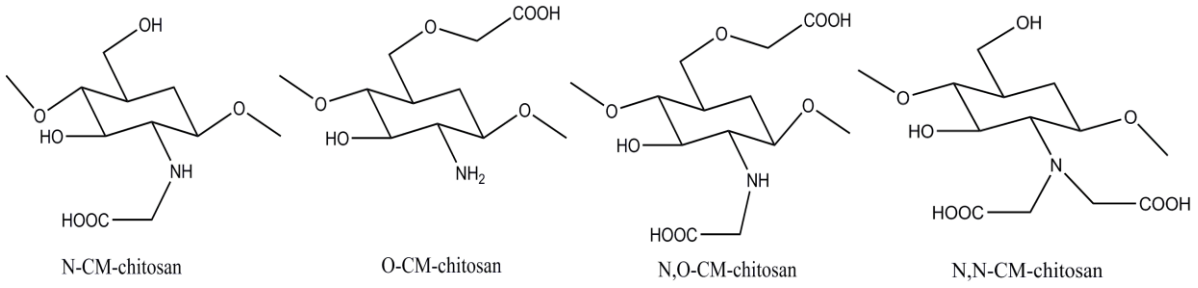
ABSTRACT: *Three compounds of carboxymethyl chitosan* (*CM-chitosan*)*; degree of substitution* (*DS*) *53.4%, 62.0% and 72.5%, were synthesized by treating chitosan with monochloroacetic acid under alkylated medium varying the reaction time and temperature. Fourier transform infrared* (*FT-IR*) *and* ^{1}*H Nuclear Magnetic Resonance* (*^{1}H NMR*) *spectroscopy were used to confirm the chemical structure of prepared products. DS was calculated by titrimetric method. According to thermogravimetric analysis* (*TGA/DTA*)*, thermal stability of chitosan decreased with increase of DS. X-ray diffraction* (*XRD*) *showed that the crystallinity/ordered arrangement of CM-chitosan decreased with increased DS. For the first time, data analysis* (*FT-IR, ^{1}H NMR, TGA/DTA and XRD*) *were carried out by varying the DS of CM-chitosan.*

KEYWORDS: *biopolymer, carboxymethyl chitosan, degree of substitution, physicochemical analysis*

I. INTRODUCTION

Chitosan is a cationic copolymer of glucosamine and N-acetylglucosamine [1]. Due to its non-toxicity, biodegradability, biocompatibility, bioadhesivity, antimicrobial activity and physicochemical and biological properties, chitosan can be applied in a variety of fields. However, the poor solubility in water and most common organic solvents limits its applications [2]. According to the literature [1, 3, 4], carboxymethylation is one of the chemical modification methods that increase the water solubility of chitosan.



Scheme 1. Types of CM-chitosan

The carboxymethylation of chitosan enhances moisture retention and adsorption properties, chelating and sorption properties, antioxidant activity, antimicrobial activity, apoptosis inhibitory activity, etc. Among the applications of CM-chitosan, sustained or controlled release drug delivery, pH responsive drug delivery and DNA delivery as permeation enhancer [1] are important. Literature [5, 6] showed that the location of substitution or type of CM-chitosan (N-, O-, N,O-, N,N-) in Scheme 1 and DS depend on the parent chitosan, reaction conditions and reaction reagents and their stoichiometry.

Numerous works [1, 7-15] have been carried out on CM-chitosan. Du and Hsieh [3] showed that longer alkalization and carboxylation time significantly improved the yield and DS of CM-chitosan products. With increasing DS, carboxymethyl substitution to the $-CH_2-OH$ at the C6 position of the chitosan was more favorable than the C3 position, since at C3 position, the substitution took place directly to the aromatic ring, which would needs more energy [16]. The studies of Abreu and Campana – Filho [7] confirmed that carboxymethylation decreased the thermal stability of chitosan. Also X-ray diffraction showed that CM-chitosan had a less ordered arrangement than parent chitosan [7]. This paper describes the preparation of three CM-

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chitosan compounds with DS 53.4%, 62.0% and 72.5%, respectively, treating chitosan with monochloroacetic acid under alkylated medium varying the reaction time and temperature. FT-IR and ^{1}H NMR spectroscopy were used to confirm the chemical structure while TGA/DTA was carried out to determine the thermal stability of CM-chitosan compounds with increasing DS. XRD analysis was used to characterize crystalline structure of three CM-chitosan compounds.

II. MATERIALS AND METHODS

2.1 Materials

Chitosan (MMW, Degree of deacetylation 75-85%) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Monochloroacetic acid was purchased from Fisher Chemical Co. (NJ, USA). The rest of the chemicals used were ACS grade.

2.2 CM-chitosan preparation

Three different degree substituted carboxymethyl chitosan compounds (CM-chitosan compound 1, CM-chitosan compound 2 and CM-chitosan compound 3) were prepared by the method of Chen and Park [16]. When preparing CM-chitosan compound 1, chitosan (10 g), sodium hydroxide (13.5 g) and solvent (100 mL) were added into a flask (500 mL) to alkalize and swell at 50°C for an hour. The temperature was maintained in a water bath (E5CS, Omron Co, IL, USA). The monochloroacetic acid (15 g) which was dissolved in isopropanol 20 mL (water/isopropanol v/v 2/8) was added into the reaction mixture for 30 min and allowed to react at 50°C. After 4 hours, the reaction was stopped by adding 70% ethyl alcohol (200 mL). The resulted solid was filtered and vacuum dried at room temperature. The product; Na salt CM-chitosan (1 g), 80% ethyl alcohol aqueous solution (100 mL) and hydrochloric acid (10 mL, 37%) were stirred for 30 min. The solid was filtered and rinsed in absolute alcohol to neutral and then vacuum dried. The product was H-form of CM-chitosan (CM-chitosan compound 1).

Both reaction temperature and time for alkalization and carboxymethylation to prepare CM-chitosan compound 2 and 3 were decided according to the method of Du and Hsiegh [3]. CM-chitosan compound 2 was prepared by 12 hour alkalization and 5 hour carboxylation at 60°C while CM-chitosan compound 3 was prepared by 12 hour alkalization at room temperature and 5 hour carboxylation at 60°C.

2.3 Characterization

FT-IR analysis was carried out on a Nicolet 6700 FT-IR spectrometer (Thermo Electron Co., WI, USA) using air. The resolution was set at 4 $cm^{-1}$with a total of 32 scans and the wave number ranges between 400 and 4000 cm^{-1}. FT-IR detects the functional groups within a compound by way of rotational, rocking, twisting and scissoring.

^{1}H NMR measurements were performed according to the method of Chen and Park [16] on a Bruker AV600 spectrometer under a static magnetic field of 600 MHz. Data analysis was carried out using TopSpin 3.1 software.

2.4 Calculation of degree of substitution (DS)

The degree of substitution of prepared CM-chitosan compounds were calculated by titrimetric method [1] using following equations,

$$\mathbf{DS = 161A / (m_{CM\text{-}CS} - 58\ A)},$$

$$\mathbf{A = V_{NaOH}\ .C_{NaOH}},$$

where $\mathbf{V}_{NaOH}$ and $\mathbf{C}_{NaOH}$ are the volume and the molarity of aqueous NaOH, respectively, $m_{CM\text{-}CS}$ is the mass of CMCS (g), and 161 and 58 are the molecular weights of the glucosamine (chitosan skeleton unit) and the carboxymethyl group, respectively.

2.5 Themogravimetric analysis (TGA/DTA)

The thermal gravimetric analysis and differential thermal analysis of the three compounds of CM-chitosan were carried out by using a TGA/DTA High Temperature 115 thermogravimetric/differential analyzer (Pyris-Diamond, PerkinElmer, MA, USA). The constant heating rate was set to 5°C/min while the temperature range was set to 50°C to 450°C. All analysis was performed under argon atmosphere.

2.6 X-ray diffraction (XRD)

Three compounds of CM-chitosan were analyzed by X-ray diffractometer (X'Pert PRO, PANalytical B.V., The Netherlands), operating at 40 kV and 20 mA with a Cu-Kα source. The diffraction intensity was measured in the range of 2Θ angles between 5° and 40°, with a step size of 0.020°, step time of 40 s and scanning speed of $0.0005°s^{-1}$.

III. Results and discussion

3.1 Determination of DS

As shown in Table 1, three different substitution values (53.4%, 62.0% and 72.5%) were obtained by the titrimetric method [3]. We assumed that the chitosan was made only from glucosamine. Even though, the chitosan that we used contain a degree of deacetylation of 75-85%. Therefore the calculated DS values are approximately correct.

3.2 Structure characterization

3.2 .1 FT-IR data analysis

FT-IR spectra of chitosan and three different degrees substituted CM-chitosan are shown in Fig.1. Among the main peaks of chitosan, O-H and N-H bond stretching around 3300 cm^{-1}, axial stretching of C-H bonds around 2900 cm^{-1}, axial stretching of C=O bonds of the acetamide groups (amide I band) at 1633 cm^{-1}, angular deformation of the N-H bonds of the amino groups at 1579 cm^{-1}, symmetric angular deformation of CH_3 at 1373 cm^{-1}, the amide II peak at 1292 cm^{-1} and the stretchings of C-O and C-O-C in the range 1186-897 cm^{-1} are important [7].

CM-chitosan products	DS	DS %
Compound 1 (1 hr alkalization and 4 hr carboxylation at 50°C)	4.35	62.0%
Compound 2 (12 hr alkalization and 5 hr carboxylation at 60°C)	5.073	72.5%
Compound 3 (12 hr alkalization at RT and 5hr carboxylation at 60°C)	3.736	53.4%

Table 1. DS values of CM-chitosan products

As shown in Fig. 1, the peak intensity centered at 3300 cm^{-1} increased with increase of DS in CM-chitosan compounds revealing that hydrophilic character of CM-chitosan increased with increase of DS. Introduction of carboxymethyl groups were confirmed by the presence of an intense peak at 1516 cm^{-1} and a moderate peak at 1377 cm^{-1}, which were due to the symmetric and asymmetric deformations of COO, respectively [7]. The peak intensity in the range 1186-897 cm^{-1} due to C-O stretch was increased with increase of DS.

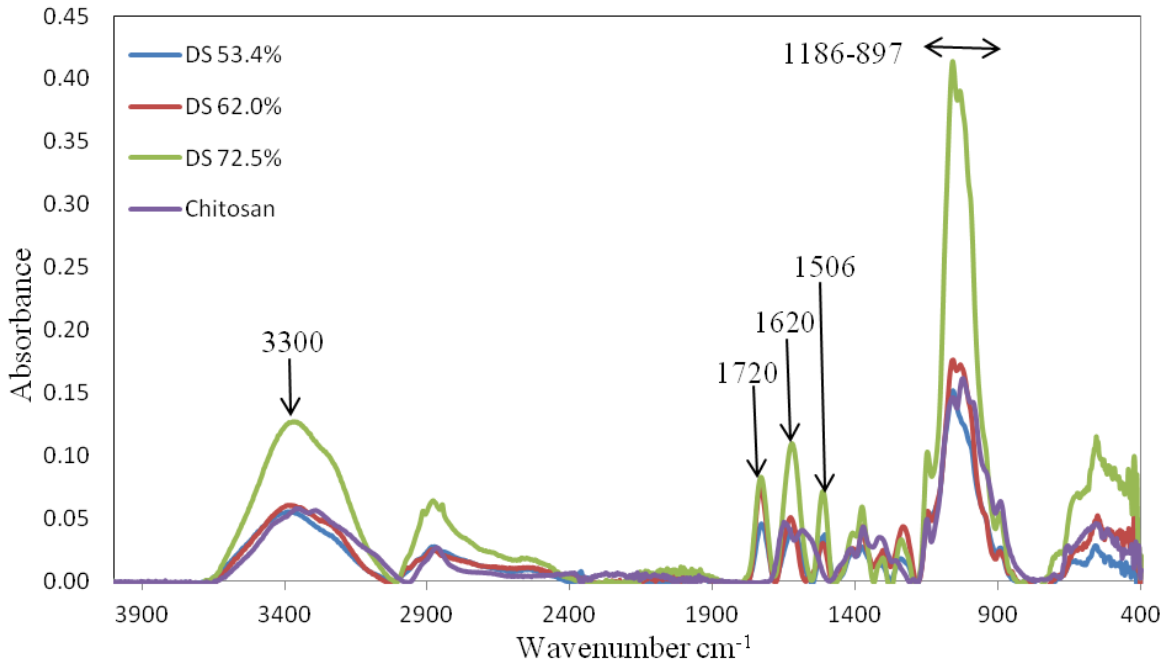


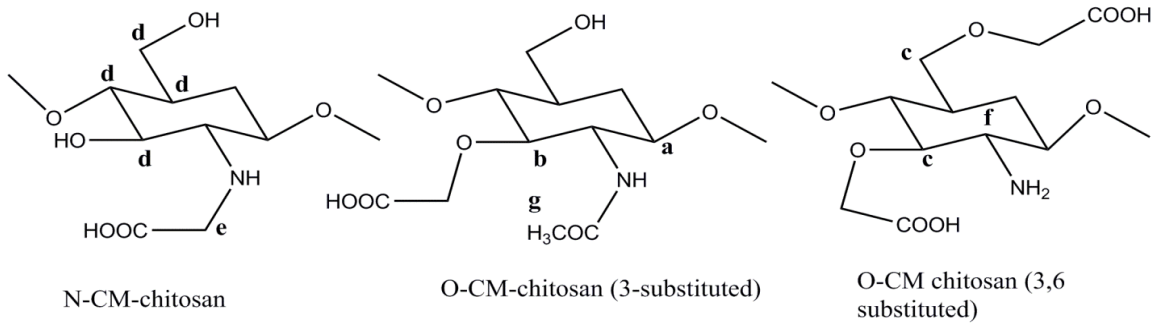
Fig. 1. FT-IR spectra of chitosan and three different degree substituted CM-chitosan

Du and Hsieh [3] stated that the peaks observed at 1061 cm^{-1} and 1032 cm^{-1} were the C-O stretches in the C2 secondary hydroxyl and the C6 primary hydroxyl, respectively. These peak intensities increased with increase of DS while the ratio of the 1061 cm^{-1}/1032 cm^{-1} increased with increase of DS. This observation further confirmed the carboxymethyl substitution to the -CH_2OH at the C6 position of the chitosan was more favorable than the C3 position with increase of DS [3].

According to the literature [16] all characteristic peaks of O-CM-chitosan [The peaks at 1720 cm^{-1}(-COOH), 870-1144 cm^{-1}(-C-O-) and 1620 and 1506 cm^{-1} (-NH_3^+) were the characteristics of O-CM-chitosan] were seen in differently substituted CM-chitosan compounds. Absorbance intensities of above mentioned peaks increased with increase of DS. In Fig. 1, intense peaks at 1622 cm^{-1} and 1377 cm^{-1} indicated that the carboxymethylation on both amino and hydroxyl groups of chitosan, which was resulted N,O-CM-chitosan [1].

3.2.2. ^{1}H NMR data analysis

All the resonances mentioned by Chen and Park [16] in the ^{1}H NMR spectrum of CM-chitosan, such as *a* (H-1A), *b* (1 proton from H-3' of 3-substituted carboxymethyl [-O-CH_2-COOD] of CM-chitosan, c (3 protons from H-6' [2 protons] and H-3' [1 proton] of 3,6- substituted carboxymethyl [-O-CH_2-COOD] of CM-chitosan, *d* (H3-6 protons), *e* (protons from N-CH_2-COOD), *f* (H-2D) and g (3 acetyl protons) were seen in the 62.0%



Scheme 2. The protons (a- g) corresponding to Fig. 2

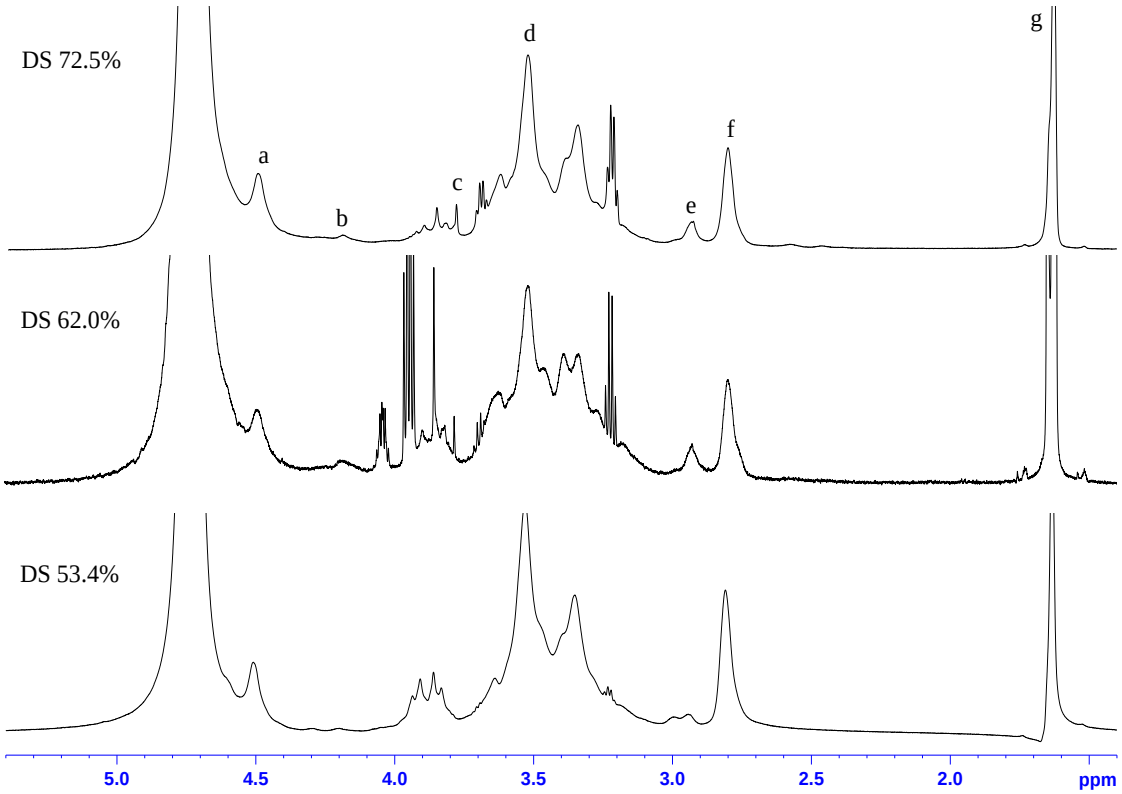


Fig. 2. ^{1}H NMR spectra of three different degree substituted CM-chitosan

degree substituted CM-chitosan product in Fig. 2 except the resonance which is due to H-1D. This may be covered by solvent peak (D_2O).

In all three spectra, c resonance had higher intensity than that of *b*. This data was consistent with the expected higher reactivity of the hydroxyl group at C6 compared with the hydroxyl group at C3 [10]. The resonance intensity of *e* was increased with increase of DS, confirming that more N-substitution would take place with increase of DS. Also O-substitution was more prevalent than N-substitution in carboxymethylation of chitosan.

3.3. Thermogravimetric (TGA/DTA)

According to TGA data in Fig. 3, the onset of degradation occurred ~ 251.5°C, 189.9°C, 169.5°C and 166°C for native chitosan, DS 53.4% CM-chitosan, DS 62.0% CM-chitosan and DS 72.5% CM-chitosan, respectively. As confirmed by Abreu and Campana-Filho [7], the thermal stability of CM-chitosan was lower than parent chitosan. Also 53.4% substituted CM-chitosan was thermally more stable than 72.5% substituted CM-chitosan. Therefore, the thermal stability of CM-chitosan decreased with increase of DS.

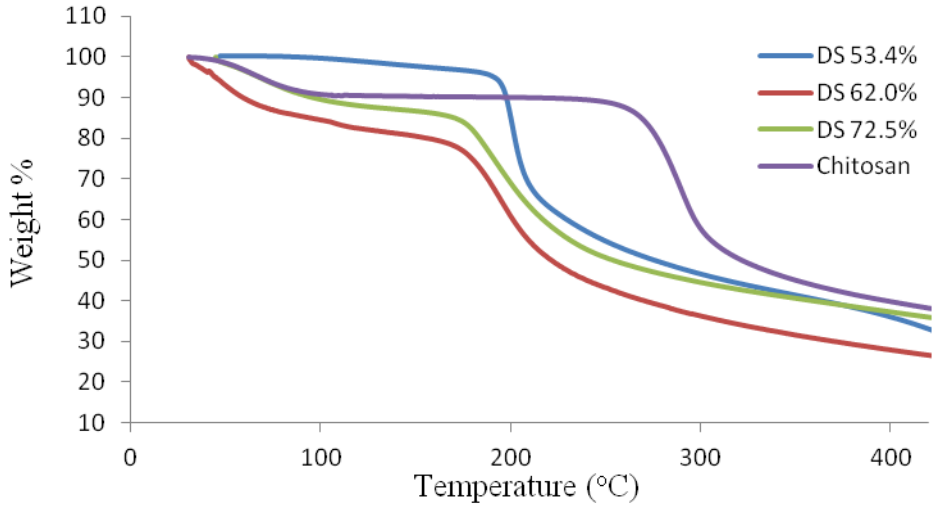


Fig. 3. TGA curves for chitosan and three different degree substituted CM-chitosan

According to the Fig. 4, chitosan showed both endothermic peak which is due to water evaporation and exothermic peak which is due to decomposition of the polymer. Endothermic peak for chitosan was centered at 46 °C with an onset of 24.8°C while exothermic peak was centered at 277.6°C with an onset of 262.5°C. But in all three CM-chitosan derivatives, it could not be able to distinguish clear decomposition peak but water evaporation peak. This concludes that carboxymethylation increases the amorphous character of chitosan.

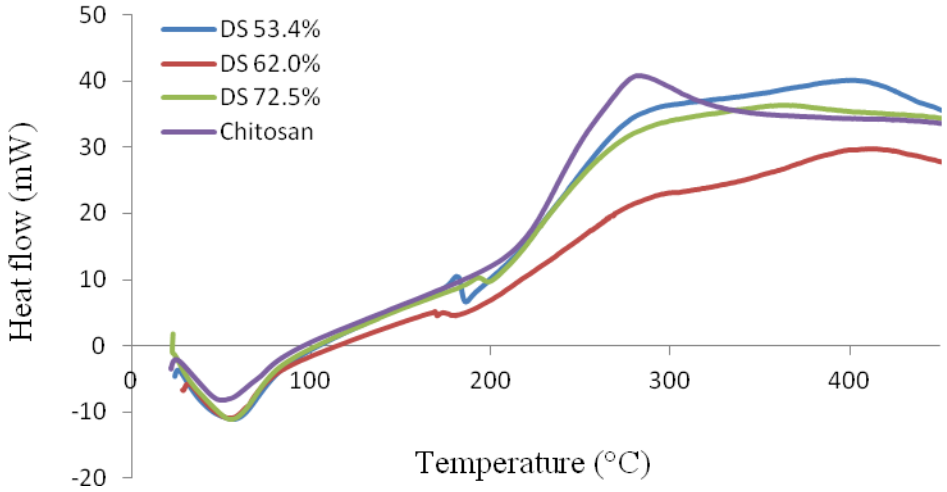


Fig. 4. DTA curves for chitosan and three different degree substituted CM-chitosan

Endothermic peak was centered at 55.8°C with an onset at 26.2°C for DS 53.4% CM-chitosan. For DS 62.0% CM-chitosan, that peak was centered at 49.4°C with an onset at 32.3°C while for DS 72.5% CM-chitosan, the peak was centered at 49.0°C with an onset at 23.4°C.

3.4 X-ray diffraction (XRD)

According to the literature [7], CM-chitosan had a less ordered arrangement compared to chitosan. Therefore CM-chitosan bears more amorphous nature. As shown in Fig. 5, in chitosan, there was an intense peak around 20° while in CM-chitosans poorly defined and less intense peaks (31.6° in DS 53.4%) were observed in the X-ray diffractograms. This was due to the less number of hydrogen bond formation between CM-chitosan molecules than chitosan. Presence of carboxymethyl groups which substitute the hydrogen atoms of the hydroxyl and amino groups of chitosan decrease the formation of hydrogen bonds.

These observations confirmed that chitosan had a more ordered arrangement than CM-chitosan products. Also chitosan had a higher degree of order than that of CM-chitosan. Therefore, the crystal structure (d spacing) of CM-chitosan was different from chitosan. According to Fig.5, X-ray diffractograms, ordered arrangement of CM-chitosan was decreased with increase of DS. Therefore the crystallinity of CM-chitosan decreased with increase of DS.

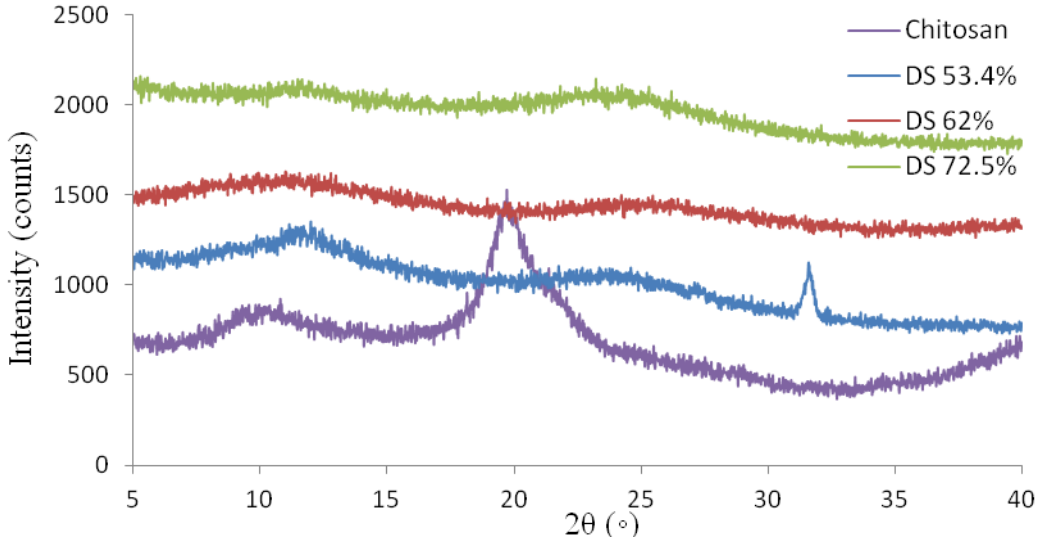


Fig. 5. XRD for chitosan and three different degree substituted CM-chitosan

IV. CONCLUSION

CM-chitosan with different DS (53.4%, 62.0% and 72.5%) were synthesized by the reaction of chitosan with monochloroacetic acid. The structure was confirmed by FTIR and ^{1}H NMR spectroscopy. According to TGA data, the onset of degradation occurred ~ 251.5°C, 189.9°C, 169.5°C and 166°C for native chitosan, DS 53.4% CM-chitosan, DS 62.0% CM-chitosan and DS 72.5% CM-chitosan, respectively. Therefore, the thermal stability of CM-chitosan decreased with increase of DS. The CM-chitosan had a different crystallinity compared to that of chitosan. Also less ordered arrangement was resulted with increase of DS of CM-chitosan.

ACKNOWLEDGEMENTS

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